

SUPPLEMENTARY INFORMATION

Vancomycin Variable Enterococci Can Give Rise to Constitutive Resistance During Antibiotic Therapy

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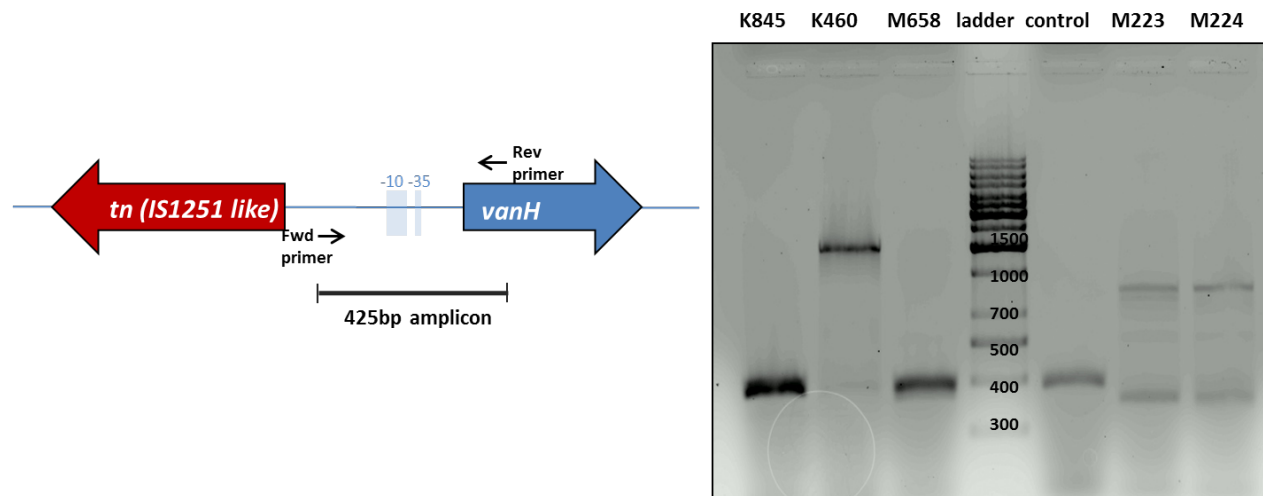


Figure S1: The PCR amplification using diagnostic primers. A) Selection of sequences for designing diagnostic primers amplifying 425bp region between the transposon and *vanH*. B) The agarose gel showing the results of PCR amplification. Control strain and K845 show expected 425bp amplicon. Strain K460 amplified unexpectedly long amplicon comparable to 1500bp band on the ladder. Strains M223 and M224 amplified for two amplicons each – one under 400bp and another around 900bp in size. M658 showed a doublet that resolved into a 425bp and a slightly smaller ~400bp band after extended electrophoresis.

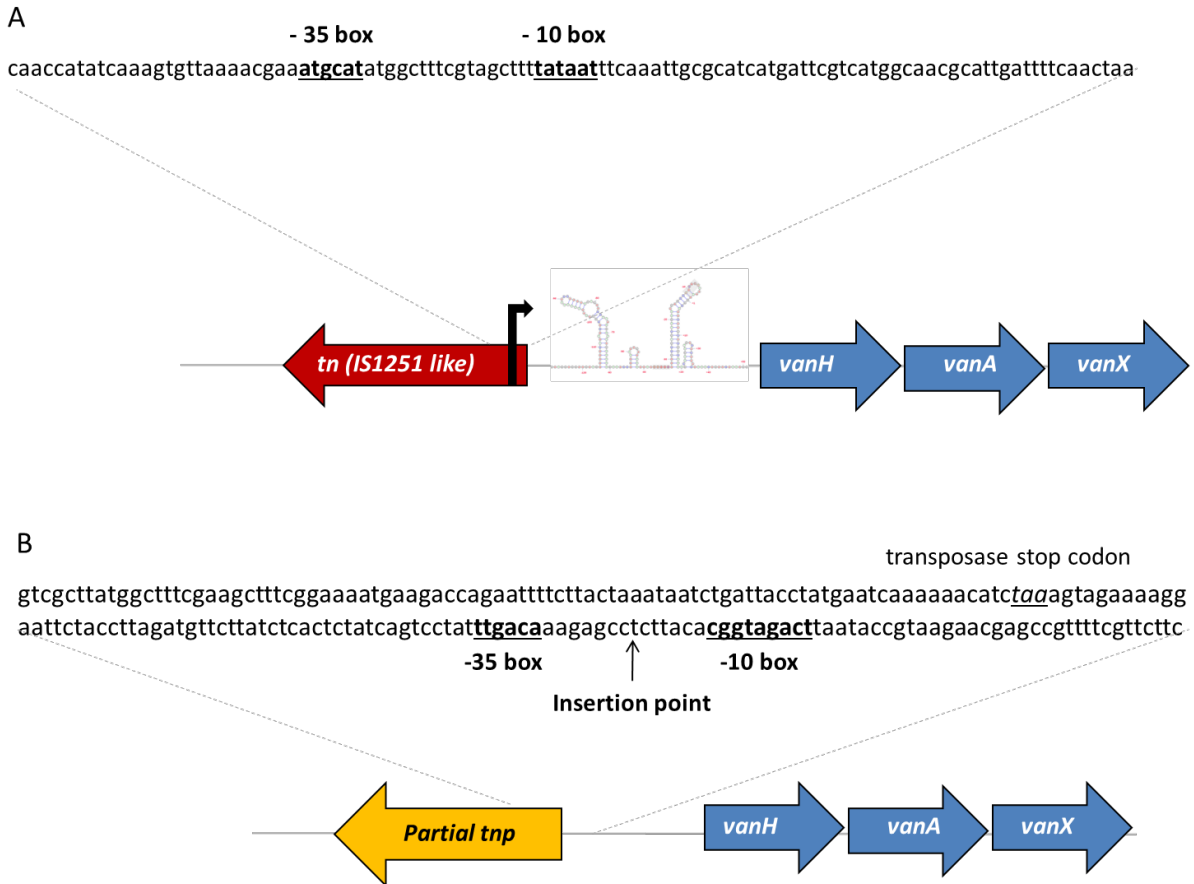


Figure S2: The results of bacterial promoter prediction software, BPRM. A) The software identifies a putative promoter in the left arm of *IS1251*-like element oriented in the divergent direction. B) Insertion of *IS1167* in K460 at position -21 creates a new hybrid promoter as determined by BPRM, where -35 box sequence is contributed by the insertion element and -10 box is supplied by the plasmid. The strategic insertion bypasses one hairpin and disrupts another thereby clearing way for the transcription of resistance cassette through the hybrid promoter.

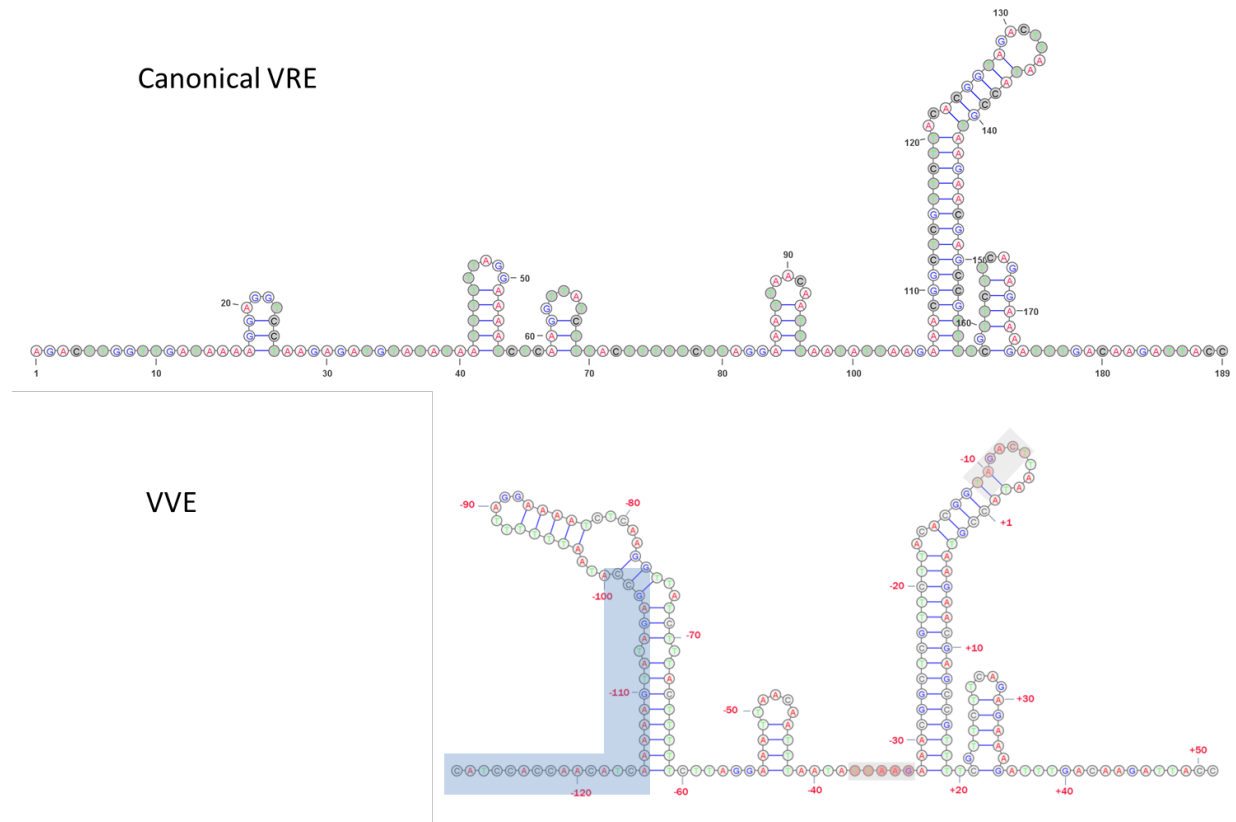


Figure S3: The difference in predicted secondary structure in VVE and the canonical VRE strains. A sequence comparison from the canonical VRE sequence (accession no. M68910) having intact *vanRS* with the VVE strains where *vanRS* is replaced by an *IS1251*-like transposon sequences. The invasion of the transposon at position -101 creates complementarity of sequences from -61 onwards in the plasmid through -115 position in the insertion element, highlighted with blue watermark. This creates an imperfect hairpin of 44bp in VVE strains, absent in the classical version of VRE.